

Original Research Article

**EFFECT OF AQUEOUS EXTRACT OF UNRIPE
SEEDS OF *CARICA PAPAYA* ON FOUR
HEMATOLOGICAL PARAMETERS OF
SALMONELLA-INDUCE ENTERIC FEVER IN
WISTAR RATS**

ABSTRACT

Aims: The study evaluates the effect of aqueous extract of the seeds of unripe *Carica papaya* on the haematological parameters of *Salmonella typhi*-induced enteric fever in Wistar rats.

Methodology: Enteric fever was induced in Swiss albino Wistar rats by intra-peritoneally inoculated of the *Salmonella typhi* isolate (0.6 ml, i.p.). Rats were divided into four groups of five rats each. First group served as negative control that was neither treated with neither standard drug nor extract. Second group as positive control that was administered 300 mg/kg body weight of Amoxicillin capsule. Groups three and four received 300 mg/kg and 500 mg/kg body weight of the aqueous extract of unripe *Carica papaya* seeds. Blood samples were analyzed for haematological parameters (PCV, HbC, TRBC, and TWBC).

Results: The *Carica papaya* extract-treated animals showed an improvement in PCV compared to the control animals. The *Carica papaya* extract-treated animals equally showed a non-significant ($P>0.05$) dose-dependent increase in the mean Hb concentration relative to that of the control animals. A non-significant ($P>0.05$) lowered TRBC was recorded for animals in the treated groups and Amoxicillin group compared to animals in the untreated group. The 300 mg/kg *Carica papaya* extract-treated animals was able to show a significantly ($P<0.05$) higher TWBC compared to the other treated animals and even the untreated group.

Conclusion: It is concluded that aqueous seeds extract of unripe *Carica papaya* is able to improve and stabilize the haematological parameters of *Salmonella typhi*-induced enteric fever.

Keywords: *Unripe seeds, Carica papaya, Salmonella typhi, Salmonella paratyphi, Enteric fever, Haematological parameters, Aqueous extract.*

11 **1. INTRODUCTION**

12 Enteric fever, an infection caused by *Salmonella enterica*, *Samonella Typhi* and *Samonella*
13 *Paratyphi* A, is common and endemic in many areas of Asian and African continents [1,2,3]
14 *Salmonella typhi* has been a major human pathogen for thousands of years thriving in
15 conditions of poor sanitations, promoted by crowding and social chaos [1,4]. Classically, its
16 presentation includes fever, malaise, abdominal pain and constipation. Untreated enteric
17 fever culminates in intestinal hemorrhage, bowel perforation, peritonitis, septicaemia and
18 death within one month of onset [5], and survivors may be left with long-term
19 neuropsychiatric complications [6]. In cases involving septicaemia, bacteria endotoxin are
20 known to be released, and this has been shown to stimulate the production of a variety of
21 inflammatory mediators which causes dramatic patho-physiological reactions in most
22 patients [7]. Such patho-physiological abnormalities may include the 'not-fully-understood'
23 thrombocytopenia and leucopenia suggested by Greisman and colleagues [8].

24 In industrialized countries the incidence of enteric fever is low and invariably associated with
25 travel to endemic locations [9]. A 2012 study by Buckle et al. [10] suggested that the regional
26 incidence of typhoid fever ranges from <0.1/100 000 in places like Europe (Eastern and
27 Central) and Asia (Central) to 724.6/100 000 in the Sub-Saharan Africa region, however, the
28 current disease burden in the African continent is unclear. This most likely can be attributed
29 to a lack of systematic research data.

30 Although the advent of antibiotics has markedly impacted on the disease, reducing the
31 frequency of it in the developed world, it however still remains endemic in developing
32 countries [10]. This is so, because, the socio-economic status and life-style of the
33 developing countries greatly favours the mode of transmission of the pathogens. Many
34 people in the developing world are used to eating food from street vendors and do not even
35 wash their hands adequately, before eating/sharing food from the same plate. Drinking

36 unpurified water and living in households that do not have good toilet facilities has not
37 helped issues either [11,12,13].

38 In the same vein, the socioeconomic status and life-style of persons in the developing
39 countries has also encouraged the use of herbs and plant materials as sources of medicine
40 against numerous diseases, which includes enteric fever. This is because herbs are
41 generally considered to be cheap, affordable [14], and considered by many locals as an
42 accessible sources of compounds free from the many side effects associated with the
43 synthetic and orthodox medicines [15].

44 *Carica papaya*, a plant which originated from South America, but now widely grown in the
45 tropical regions, represent one of the very popular medicinal plants that has acquired wide
46 use in the treatment of many tropical diseases [16]. While the fruits of *Carica papaya* have
47 been shown to contain compounds such as vitamin C and vitamin A [17], previous
48 phytochemical studies on the seeds has shown that it also contains polyphenols, alkaloids,
49 flavonoids [18], phytate, tannin, protein, negligible oxalate [19,20], phosphatidylcholine and
50 cardiolipin (both phospholipids), fatty acids, carpaine, beta-sitosterol, benzyl glucosinolate,
51 benzyl isothiocyanate, caricin, enzyme myrosin, and proteinases (chymopapain, glycyl
52 endopeptidase, caricain, and papain) [21,22].

53 Studies by Yismaw *et al.* [23] as well as by Krishna *et al.*, [16]; Afolayan [24] and Osato *et*
54 *al.*, [25] on micro bacterial properties of *Carica papaya* have also shown in their separate
55 studies that the seeds possesses inhibitory properties against human enteric pathogens
56 such as *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*,
57 *Pseudomonas aeruginosa*, *Bacillus cereus* and other bacteria *in vitro*

58 Having established that *Carica papaya* possess inhibitory properties on the *Salmonella*
59 *typhi* pathogen [16,23,24,25] and with previous data on human studies reported by
60 Wealtheral *et al.*, [26], Cheesbrough [27] and Hoffbrand *et al.* [28] indicating that typhoid and

61 paratyphoid fevers are associated with a distortion of haematological parameters (by
62 precipitating anaemia as well as a general decrease in total white blood cell count), this
63 study is therefore intended to evaluate the effect of aqueous extract of unripe seeds of
64 *Carica papaya* on haematological parameters (PCV, HbC, RBC, and TWBC) in an animal
65 model of *Salmonella*-induced enteric fever.

66

67 **2. MATERIAL AND METHODS**

68

69 **2.1 Collection of unripe *Carica papaya* fruits and seeds.**

70 Unripe *Carica papaya* fruits were purchased from Abraka main market and were opened
71 with a knife and the seeds collected. The unripe seeds of *Carica papaya* were authenticated
72 at the Botany Department, Delta State University, Abraka, Nigeria. The weight of the
73 collected seeds was recorded using a weighing balance.

74

75 **2.2 Preparation of crude extracts**

76 The *Carica papaya* seeds were air-dried until there was no water in them. The dried *Carica*
77 *papaya* seeds were grinded with a machine. The grinded *Carica papaya* seed weighing
78 170g was soaked in 2000ml of distilled water and was gently, but continuously agitated for 72
79 hours. The mixture was then filtered using dried Whatman's filter paper and the filtrate
80 concentrated using a rotary evaporator to form a paste-like solid (32 g).

81

82 **2.3 Phytochemical screening**

83 The aqueous *Carica papaya* seeds extract was subjected to qualitative screening to
84 identify the bio-chemicals constituents present in the extract. The simple and standard
85 qualitative methods described by Trease and Evans [29] were used.

86

87 **2.4 Animal**

88 Twenty (20) Swiss Albino Wistar rats of both sexes, weighing 150-230 g used for the study
89 was obtained from the breeding colony in the Animal House of the Department of Anatomy,
90 School of Basic Medical Sciences, University of Benin, Benin City, Nigeria. The animals
91 were kept in well ventilated cages at room temperature ($24 \pm 2^{\circ}\text{C}$), with a 12:12 hour
92 light/dark cycle, and were allowed free access to clean drinking water and growers mash
93 (Guinea Food Nigeria Ltd). The animals were acclimatized for two weeks and received
94 humane care in compliance with the ethical guide approved by the College of Health
95 Sciences, Delta State University, Abraka, Delta State, Nigeria, in accordance with the NIH
96 guidelines for the care and use of laboratory animals.

97

98 **2.5 Collection of bacteria isolates**

99 Pure cultures of *Salmonella typhi* bacteria were collect from the main laboratory of the
100 Department of Microbiology, Delta State University, Abraka, Nigeria. The isolated
101 *Salmonella typhi* was sub-cultured in a McCartney bottle containing nutrient broth and were
102 screened for β -lactamase production, using Livermore and Brown [30] model. Isolates that
103 were positively confirmed were kept in MacConkey agar and stored in a refrigerator (40°C) in
104 the laboratory in the Department of Pharmacology and Therapeutics, Delta State University,
105 Abraka, Nigeria, before inoculation.

106

107 **2.6 Inoculation of typhoid fever**

108 The rats were fasted for 18hrs before the intra-peritoneal administration of 0.6ml of the
109 *Salmonella typhi* isolate into each induced rats. Seventy-two (72) hours following the
110 inoculation, the induced rats were tested (Widal test) to confirm the presence of enteric fever
111 in the rats

112

113 **2.7 Widal test**

114 The Widal tube agglutination test (Sanofi Diagnostics Pasteur, Marnes la Coquette, France)
115 was performed according to the manufacturer's instructions by a single operator blind to the
116 patient's diagnosis. Briefly, serial tube dilutions of sera were made in 0.85% saline, starting
117 at a dilution of 1/100. Standard preparations of *Salmonella* O and H antigens were added,
118 and the tubes were incubated at 37°C for 1 h. The tubes were centrifuged for 5 min, and
119 agglutination was determined by visual inspection. The Widal TO or TH titer was taken as
120 the highest dilution of serum with visible agglutination.

121

122 **2.8 Experimental design**

123 The twenty (20) rats with an average weight of 150-230 g were randomly divided into four
124 groups with each group having five rats.

125 **Group I** (Negative Control) Induced with *Salmonella typhi* isolate and fed with rat
126 chow and water.

127 **Group II** (Positive Control) Induced with *Salmonella typhi* isolate and administered
128 300 mg/kg of the Amoxicillin capsule and fed with rat chow and water.

129 **Group III** Induced with *Salmonella typhi* isolate and administered 300 mg/kg of the
130 aqueous extract of unripe *Carica papaya* seeds and fed with rat chow and
131 water.

132 **Group IV** Induced with *Salmonella typhi* isolate and administered 500 mg/kg of the
133 aqueous extract of unripe *Carica papaya* seeds and fed with rat chow and
134 water.

135 The extracts for each of the treated groups as well as the Amoxicillin capsule for the positive
136 control group, was administered orally with an oral cannula once daily between the hours of
137 08:00 am and 09:00 am for a period of 14 days (2 weeks) with the weight of the rats taken
138 every day for the periods of the study.

139

140 **2.9 Sample collection**

141 Following an overnight fast, the rats were sacrificed in the morning by inhalation anesthesia
142 using chloroform. A laparotomy was carried out to expose the major blood vessels and
143 internal organs. 5ml of blood was collected with the aid of a needle and syringe by cardio-
144 puncture from each rat and the blood from each rat placed into well labeled clean collecting
145 specimen bottles (EDTA anti-coagulant)

146

147 **2.10 Packed Cell Volume (PCV) Determination**

148 Packed cell volume was determined by the microhaematocrit method according to the
149 NCCLS Document H7-A2, Procedure (NCCLS Document H7-A3, 2000).

150

151 **2.11 Haemoglobin Concentration (HB) Determination**

152 Haemoglobin concentration was determined by the method of Agbede *et.al.*, [31].

153

154 **2.12 Determination Red Blood Cell Count**

155 Red blood count (RBC) was determined by the improved Neubauer's counting chamber
156 according to the method of Blaxhall and Daisley [32].

157

158 **2.13 Determination of Total White Blood Cell (TWBC) Count**

159 The total white blood cell count was determined by the visual method as described by Dacie
160 and Lewis [33].

161

162 **2.14 Statistical Analyses**

163 All data obtained were expressed as Mean $\pm$ SEM (standard error of mean) of sample size,
164 $n=5$. Significant difference between control and experimental groups were obtained by
165 student's t-test using statistical package for social science (SPSS version 16). P-values
166 <0.05 were considered significant.

167

168

169 **3. RESULTS AND DISCUSSION**

170

171 **Table 1:** Qualitative phytochemical constituents of the aqueous extracts of the
172 seeds of unripe *Carica papaya*.

Constituents	Aqueous extract
Phenols	+
Tannin	+
Saponins	+
Anthocyanosides	+
Anthraquinones	+
Reducing sugars	+
Fat	+
Protein	+
Carbohydrate	+
+ represents present; - represents absent	

173

174

175 **Table 2:** Effect of aqueous ripe *Carica papaya* seeds on body weight of Wistar
176 rat

Groups	Initial weight (g)	Final weight (g)
Induced and not treated (Control)	171±3.30	152±6.79
Amoxicillin capsule (300 mg/kg)	187±6.97	164±2.58
Unripe <i>Carica papaya</i> (300 mg/kg)	209±8.12	216±7.03
Unripe <i>Carica papaya</i> (500 mg/kg)	223±5.50	227±5.02

177 Values are presented as means ± SEM; * denotes significance at $P < 0.05$ compared with
178 body weight before experiment (n=5)

179

180 **Table 3:** Effect of aqueous extract of unripe seeds of *Carica Papaya* on Widal
181 test, showing initial Widal test results before treatment with either
182 extract or standard drug.

Groups		Salmonella antibody tyters			
		A	B	C	D
Induced and not treated (Control)	O (Somatic)	1:160	1:160	1:160	1:160
	H (Non-Somatic)	1:160	1:160	1:160	1:160
Amoxicillin capsule (300 mg/kg)	O (Somatic)	1:160	1:160	1:160	1:160
	H (Non-Somatic)	1:160	1:160	1:160	1:160
Unripe <i>Carica papaya</i> (300 mg/kg)	O (Somatic)	1:160	1:160	1:160	1:160
	H (Non-Somatic)	1:160	1:160	1:160	1:160

Unripe <i>Carica papaya</i> (500 mg/kg)	O (Somatic)	1:160	1:160	1:160	1:160
	H (Non-Somatic)	1:160	1:160	1:160	1:160

183

184

185 **Table 4: Effect of aqueous extract of unripe seeds of *Carica Papaya* on Widal**
186 **test, showing final Widal test result after treatment with either extract**
187 **or standard drug.**

Groups		<i>Salmonella</i> antigen tyters			
		A	B	C	D
Induced and not treated (Control)	O (Somatic)	1:160	1:160	1:160	1:160
	H (Non-Somatic)	1:160	1:160	1:160	1:160
Amoxicillin capsule (300 mg/kg)	O (Somatic)	1:160	1:20	1:80	0
	H (Non-Somatic)	1:80	0	0	1:80
Unripe <i>Carica papaya</i> (300 mg/kg)	O (Somatic)	1:20	1:20	1:20	0
	H (Non-Somatic)	1:80	1:80	1:80	1:80
Unripe <i>Carica papaya</i> (500 mg/kg)	O (Somatic)	1:160	1:80	1:160	1:160
	H (Non-Somatic)	1:80	0	1:80	0

188 *0 = Negative reaction*

189 *1:20 = Insignificant titre*

190 *1:80 = Mild positive for Salmonella*

191 1:160 = Strongly positive for *Salmonella*

192 A – C = *Paratyphi* antigen

193 D = *Typhi* antigen.

194

195 **Table 5:** Effect of aqueous extract of unripe seeds of *Carica Papaya* on the
196 haematological parameters of *Salmonella*-induce enteric fever in
197 Wistar rats

Groups	PCV (%)	Hb (g/dl)	RBC ($\times 10^6/\text{mm}^3$)	TWBC ($\times 10^3/\text{mm}^3$)
Induced and not treated (Control)	28.2 $\pm$ 1.54	9.31 $\pm$ 0.38	4.13 $\pm$ 0.54	7.62 $\pm$ 0.25
Amoxicillin capsule (300 mg/kg)	34.6 $\pm$ 2.20	11.93 $\pm$ 0.54	6.21 $\pm$ 0.38	5.02 $\pm$ 0.15
Unripe <i>Carica papaya</i> (300 mg/kg)	43.4 $\pm$ 0.52	12.86 $\pm$ 0.41	6.42 $\pm$ 0.10	4.01 $\pm$ 0.27*
Unripe <i>Carica papaya</i> (500 mg/kg)	41.4 $\pm$ 0.36	13.92 $\pm$ 0.19	6.98 $\pm$ 0.46	4.76 $\pm$ 0.04

198 Values are presented as means $\pm$ SEM; * denotes significance at $P < 0.05$ compared with
199 control and experimental groups (n=5)

200

201 Documented and undocumented traditional evidence abounds of the use of most of the
202 vegetative parts of *Carica papaya* to treat enteric fever in some parts of Nigeria [34,35].

203 The study was undertaken to evaluate the effect of aqueous extract of unripe seeds of
204 *Carica papaya* on four hematological parameters of *Salmonella*-induce enteric fever in an
205 animal model. Besides carrying out a qualitative phytochemical analysis of the unripe seeds
206 of *Carica papaya*, the changes in weight and in the haematological parameters (PCV, Hb,
207 RBC, and TWBC) of the different animal groups were observed, just as the initial and final

208 level of bacteremia in the different animal groups was also observed as part of the design for
209 his study.

210

211 The result of the qualitative phytochemical screening as shown in Table 1 revealed the
212 presence of compounds such as phenols, tannins, saponins, anthocyanosides,
213 anthraquinones and reducing sugars. Tannins found in extracts of *Carica papaya*, through
214 its metal chelating activity has been shown to possess antibacterial properties, just as
215 phenolic compounds and the terpenoids present in extracts of seeds of *Carica papaya* has
216 previously been shown to possess strong antimicrobial activity against *Salmonella typhi* [25].

217

218 In Table 2, the control groups revealed a continuous decrease in the body weight as the
219 week went by for both the negative and positive controls. However, there was a continuous
220 increase in weight observed in the extract treated groups, where there was an initial increase
221 in body weight, then a decrease in weight in the second week, which was subsequently
222 followed by an increase in body weight. This increase in body weight may be attributed to
223 the nutritional effects (presence of carbohydrates, fat and protein) of the extracts of the
224 seeds of unripe *Carica papaya*, and is supported by earlier findings by Krishna *et al.* [16].

225

226 Comparing the control group of animals to those treated with 300mg/kg of Amoxicillin, as
227 shown in Tables 3 and 4, revealed that there was a gradual improvement in their paratyphi
228 antibodies of those treated with 300mg/kg of Amoxicillin. The Somatic antibodies gave a
229 negative reaction on the typhi antibodies compared to those of the control animals. As for
230 the Non-Somatic antibodies, a negative titre was also obtained at B and C, with mild titre
231 obtained on A and D, compared to control animals. However, following treatment with 300
232 mg/kg aqueous extract of unripe seeds of *Carica papaya*, there was an appreciable
233 reduction in the paratyphi antibodies A – C, while the typhi antibodies showed negative
234 reaction in comparison to the control. With the 500 mg/kg aqueous extract of unripe seeds of

235 *Carica papaya*, there was no change in reaction seen in both paratyphi and typhi antibodies
236 on the Somatic antibodies. However, the Non-Somatic antibody (H) was significantly
237 reduced on the paratyphi A – C, with negative reaction on the typhi antibodies (D). We can
238 therefore conclude that, compared to the animals in the control group, those animals treated
239 with either 300 mg/kg Amoxicillin or 300 mg/kg aqueous extract of unripe seeds of *Carica*
240 *papaya*, demonstrated better clearance abilities of paratyphi and typhi antibodies. A less
241 better clearance ability of paratyphi and typhi antibodies was observed with the 500 mg/kg
242 aqueous extract of unripe seeds of *Carica papaya* compared to the 300 mg/kg aqueous
243 extract of unripe seeds of *Carica papaya*, especially on the Somatic antibodies, while a
244 better clearance was observed on the Non-Somatic (H) antibodies. While the low clearance
245 ability of paratyphi and typhi antibodies demonstrated by the 500 mg/kg aqueous extract
246 may be explained by factors such as the masking potentials of some particles when in high
247 concentrations, this observation with the 300 mg/kg aqueous extract of unripe seeds of
248 *Carica papaya* could just mean that 300 mg/kg is the optimal concentration for best activity
249 for aqueous extract of unripe seeds of *Carica papaya*. However, the demonstration that
250 extract of seeds of *Carica papaya* possesses the ability to ameliorate *Salmonella*-induced
251 enteric fever is supported by previous findings by Osato *et al.* [25] and Mulu and Okoye [36].
252

253 In Table 5, the changes in haematological parameters of animals treated with aqueous
254 extract of unripe seeds of *Carica papaya* were compared to those of the control animals, as
255 well as those treated with standard drugs. With regards to the PCV, there was a non-
256 significant ($p > 0.05$) increase in the PCV of the unripe *Carica papaya* seeds extract-treated
257 animals (300 mg/kg = $43.4 \pm 1.17\%$; 500 mg/kg = $41.4 \pm 0.81\%$), relative to that of the
258 untreated ($28.2 \pm 3.46\%$) and Amoxicillin-treated ($34.6 \pm 4.92\%$) animals (Table 5). The result
259 also showed that the 300 mg/kg aqueous extract did improve the PCV more than the 500
260 mg/kg aqueous extract. This could just be that the 300 mg/kg aqueous extract represents
261 the optimal concentration of the extract for improving PCV.

262 The Hb also showed a non-significant ($p > 0.05$) dose-dependent increase in the mean Hb
263 concentration for the unripe *Carica papaya* seeds extract-treated animals (300 mg/kg =
264 12.86 ± 0.91 ; 500 mg/kg = 13.92 ± 0.42), relative to that of the control (9.31 ± 0.86) and
265 Amoxicillin-treated (11.93 ± 1.21) (Table 5). However the mean Hb concentration of the
266 Amoxicillin-treated animals (300 mg/kg) was found to be lower than those of the extract-
267 treated animals.

268

269 The total RBC count of animals in each of the treated groups – Amoxicillin-treated (300
270 mg/kg) (6.21 ± 0.85), unripe *Carica papaya* seeds-treated (300mg/kg) (6.42 ± 0.22), and
271 (500mg/kg) (6.98 ± 1.02) were observed to be higher, relative to those in the control groups of
272 animals (4.13 ± 1.20), however, differences were found to be non-significant ($P > 0.05$) (Table
273 5).

274

275 The result from the study on changes in total WBC shows that whereas the values for the
276 treated animal – Amoxicillin-treated (300 mg/kg) (5.02 ± 0.34), unripe *Carica papaya* seeds-
277 treated (300mg/kg) ($4.01 \pm 0.60^*$) and (500mg/kg) (4.76 ± 0.10), were observed to be lower
278 compared to those of control animals (4.62 ± 0.55). While the decrease observed with the
279 300 mg/kg unripe *Carica papaya* seeds-treated animals was shown to be significant ($p <$
280 0.05) compared with the other treated groups of animal, the unripe *Carica papaya* seeds-
281 treated (300mg/kg) ($4.01 \pm 0.60^*$) and (500mg/kg) (4.76 ± 0.10), showed a much improved
282 result than the Amoxicillin-treated (300 mg/kg) (5.02 ± 0.34) animals.

283

284 The decrease in mean levels in PCV, Hb, RBC, with an increase in mean level in TWBC
285 observed in the untreated (control) group of animals, is in line with symptoms of *Salmonella*-
286 induced enteric fever [37]. This may be due to the increased bacteremia which causes
287 decreased haematopoiesis and increased RBC destruction. In the same vein, the increase
288 in mean levels in PCV, Hb, and RBC, with a corresponding decrease in mean levels in

289 TWBC seen in the Amoxicillin-treated (300 mg/kg) and the unripe *Carica papaya* seeds
290 extract-treated (300 mg/kg and 500 mg/kg) animals could also be ascribed to an
291 improvement in haemopoietic/bone marrow activity. Specifically, the significantly ($p < 0.05$)
292 decrease in mean level in TWBC following the administration of 300 mg/kg aqueous extract
293 of unripe seeds of *Carica papaya* may be due to the optimum inhibitory bactericidal activity
294 of the extract on *Salmonella typhi* [38]. These observations are comparable to findings by
295 Olatunji [39] who also studied the effect of aqueous extract of *Hibiscus sabdariffa*-petals on
296 the haematological parameters of rats.

297

298

299 **4. CONCLUSION**

300

301 The result of the study has shown that aqueous extract of unripe seeds of *Carica papaya*
302 can possibly improve some haematological parameters (PCV, Hb, RBC, and TWBC) in
303 *Salmonella*-induced enteric fever. This situation has been suggested to be due to the
304 observed inhibitory bactericidal activity of the extract on *Salmonella typhi* (as demonstrated
305 by the decrease in mean levels in TWBC seen in the unripe *Carica papaya* seeds extract-
306 treated animals) as well as an improvement in haemopoietic/bone marrow activity promoted
307 by the extract. This finding supports the use by local medicine practitioners of the unripe
308 seeds of *Carica papaya* in the treatment of anaemic condition induced by *Salmonella typhi*
309 infection.

310

311 **ETHICAL APPROVAL (WHERE EVER APPLICABLE)**

312 I hereby declare that "Principles of laboratory animal care" (NIH publication no. 85-23,
313 revised 1985) were followed, as well as specific national laws where applicable. All

314 experiments were examined and approved by the Delta State University's ethical committee
315 for the use of laboratory animals.

316 I hereby declare that all experiments have been examined and approved by the appropriate
317 ethics committee and have therefore been performed in accordance with the ethical
318 standards laid down in the 1964 Declaration of Helsinki.

319

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413

414 **ABBREVIATIONS**

415 PCV - Pack cell volume

416 TRBC - Total red blood cell

417 HbC - Haemoglobin Concentration

418 TWBC - Total white blood cell